SEROLOGICAL STUDIES OF THE ROOT-NODULE BACTERIA.

III. TESTS OF NEIGHBOURING STRAINS OF THE SAME SPECIES.

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Introduction.

The results of many investigations have shown that the root-nodule bacteria are characterized by considerable cultural, biochemical and serological variation within the species, and by differing ability to fix nitrogen in association with a particular host plant. But it has been exceptional for the exact locality of the host plant from which the culture was first isolated to be reported; frequently it is stated that the strains were obtained from widespread areas and in other cases they have been supplied from collections or obtained from commercial cultures. The extent of similarity or differences that occur between isolates from contiguous plants has seldom been indicated; there is some information as to strains isolated from the single plant and within the one nodule, but even here more information seems to be required.

A. *Strains from Separate Plants.*—Helz, Baldwin and Fred (1927) included in their cultures seven strains obtained from pea plants growing in Urbana, Illinois, U.S.A. No more detailed information was supplied as to the relative location of the host plants and, as judged by growth of inoculated pea plants, there was no striking difference between the strains. Eckhardt, Baldwin and Fred (1931) included four strains of *Rhizobium lupini* from Madison, Wisconsin, U.S.A., and three from Hancock in the same state. Growth and changes in H-ion concentration were recorded for many sugar media and some of the strains coming from the same area showed differences in behaviour, especially in amount of growth. However, in the absence of information as to reproducibility of the results with the same strain, the significance of such differences is not established. Tested against *Lupinus albus* L., the three Hancock strains differed as indicated below:

	Strain.			
	811	812	813	Control.
Appearance of plants	Very good.	Good.	Fair.	Poor.
Dry wt. per plant (gm.)	0·820	0·619	0·478	0·395
Per cent. nitrogen	2·93	3·19	3·21	1·79

Leonard and Dodson (1933) studied six isolates from plants of Austrian winter pea (*Pisum sativum* L. var. *arvense* Poir.), which were evidently growing in the one farm area, and found that they differed in the level of efficiency of fixation with this host. Since three cultures obtained from plants of Louisiana vetch (*Vicia ludoviciana* Nutt) which were growing nearby, all proved ineffective with the Austrian winter pea in greenhouse trials, the authors postulated these plants as the source of the strains which were proving ineffective with the cultivated pea. The same workers reported beneficial results from seed inoculation with tested strains, even though the plants were growing in areas known to harbour inefficient strains.

Wright, Sarles and Holst (1930) briefly reported a study of 156 strains of *Rhizobium japonicum* isolated from soils in which soybeans had been growing. Without giving details these authors stated that they found two main cultural and six serological

groups and that there was a marked tendency for strains of any one soil to fall into the same serological and cultural group. However, the same group could contain strains from more than one area. It seems possible that this degree of homogeneity within an area is more apparent than real and had flagellar and somatic agglutination been distinguished further subdivision might have occurred amongst strains obtained from the same soil. In this connection it is relevant to compare the more detailed subdivision of *Rhizobium meliloti* and *Rh. trifolii* that has followed from the recognition of the two types of agglutination as reported in the two earlier papers of this series (Vincent, 1941, 1942). Where the strains used in these studies were obtained from nearby areas it was observed that in a number of cases they were serologically distinct. Examples have been abstracted and are set out in Tables 1 and 2 below.

TABLE 1.
Reactions of Some Strains of Rhizobium meliloti (*Vincent, 1941*).

			Reactions with Test Sera.						
			Flagellar.		Somatic.				
Collection No.	Host Plant.	Source.	47 Ab.	27 bC.	47 I, III.	27 I, IV.	74 II, VI.	102 III, V.	66 II, VII.
62	M.H.D.	Roseworthy, S.Aust. ..	1	4	2	4	—	—	—
74	M.S.	,, ,, ..	1	4	—	—	3	—	1
24	M.S.	Leeton, N.S.W.	1	4	2	4	—	—	—
25	M.S.	,, ,, ...	3	1	2	2	—	—	—

Key to host species: M.H.D. = *Medicago hispida* Gaertn. var. *denticulata* Urb.; M.S. = *Medicago sativa* L.
Key to reactions: 4 = positive at 1/3200 or higher; 3 = positive at 1/800; 2 = positive at 1/200; 1 = positive at 1/50; ± = inconsistent at 1/50; − = no reaction at 1/50.
The signs: Ab, bC and I-VII are those applied in the previous paper to represent the antigenic constitution of strains of *Rhizobium meliloti*.

TABLE 2.
Reactions of Some Strains of Rhizobium trifolii (*Vincent, 1942*).

			Reactions with Test Sera.				
			Flagellar.		Somatic.		
Collection No.	Host Plant.	Source.	36 A.	46 B.	36 I.	61 II.	46 III.
108	T.S.	Manildra, N.S.W.	3	—	4	—	—
35	T.S.	,, ,, ...	3	—	—	4	—
114	T.S.	,, ,, ...	2	—	—	—	—
156	T.G.	Glen Innes, N.S.W.	3	—	—	—	—
159	T.S.	,, ,, ,, ..	—	—	—	2	1
161	T.P.	,, ,, ,, ..	—	—	—	—	—
153	T.G.	Goonoo Goonoo, N.S.W. ..	2	—	—	—	4
142	T.G.	,, ,, ,, ..	—	—	—	—	—

Key to host species: T.G. = *Trifolium glomeratum* L.; T.P. = *T. pratense* L.; T.S. = *T. subterraneum* L.
Key to reactions: As in Table 1.
The signs A, B and I-III are those applied in the previous paper to represent the antigenic constitution of strains of *Rhizobium trifolii*.

B. *Strains from the Same Plant.*—Dunham and Baldwin (1931) briefly review earlier reports on the simultaneous presence of two or more strains of nodule bacteria on a single host plant. Particularly they refer to the use of serological methods of distinguishing strains and note that several workers (Bialosuknia and Klott, Okawara and Yoshida, and Jimbo) have found more than one strain. On the other hand, it is recorded that Israilsky failed to find more than one strain on a single plant. The same authors report that Sarles with *Rhizobium japonicum* and Gray with *Rh. meliloti* had distinguished two strains within the single nodule. It was considered that coalescence of adjacently-developing nodules might explain this evidently unusual condition. Dunham and Baldwin themselves were able experimentally to obtain double infection with two

distinct strains of *Rhizobium meliloti* and found that generally this was more easily effected if the two cultures were provided simultaneously. It seemed that previous invasion by a strain checked or prevented invasion by another. They found no case of double infection within the one nodule. Nicol and Thornton (1941) have recently presented much more evidence on the points raised by Dunham and Baldwin. They found that in peas and soybeans the "nodule-producing capacity" of the plant may be partially or wholly satisfied by the nodules produced within the first few weeks and that this might explain subsequent failure of another strain to enter. These workers were unable to obtain any evidence supporting the idea of a resistance developed to a second strain. In cases where one strain tended to form more nodules, and these more rapidly, than another it seemed that this was due to the rapid growth made by the successful strain which led to its "dominance" in the flora surrounding the root-hairs.

Technique for the Recognition of Strains.

Whilst, from a strictly practical point of view, the most important characteristic of a strain of *Rhizobium* is its ability to fix nitrogen in association with a particular host, tests of this nature occupy a good deal of time and apparatus, and the number of strains which can be tested is thereby limited. Moreover, it is questionable whether, in the matter of fundamental differences or similarities, the efficiency level of a strain is an adequate criterion. This is particularly suggested by the results of Allen and Baldwin (1931), which indicate that the efficiency level can be affected quite readily by repeated exposure to a host plant. According to Fred, Baldwin and McCoy (1932), the same workers found that other properties, including serological, remained unchanged. Such antigenic constancy going along with varying effectiveness is in accord with the considerable body of evidence accumulated by medical and veterinary bacteriologists, and is supported by those who have used serological methods for the study of *Rhizobium*. Experience in this laboratory over the last two years emphasizes the same fact. The agreement between complementary antigen/antiserum tests (position of antigen and antiserum reversed) reported in the two earlier papers as well as the result of a repeated animal inoculation with one strain, show that the reaction is qualitatively independent of the individual animal. Tests which have been carried out after continued cultivation and subculturing on laboratory media have agreed closely with earlier tests, and the reactions of strains re-isolated from inoculated host plants have been quite unaltered.

For these reasons it is felt that the agglutination reaction reflects some fundamental and relatively constant characteristic of the bacterial cell and provides a ready and reliable means for the recognition and separation of strains. The methods used are essentially those described in the earlier papers for the recognition of flagellar (*H*) and somatic (*O*) reactions and the sera are those already used for *Rhizobium meliloti* and *Rh. trifolii*.

In presenting results, letters (a, b, etc.) are applied to designate groupings of similar, though not necessarily identical, reactions within an individual experiment. It is emphasized that such groupings represent a temporary expedient for convenience in discussion, and that the symbols used have no relationship between experiments.

RESULTS.

A. *Strains isolated from Separate Plants.*

Experiment 1.—Isolations from Medicago minima.

Plants were collected from the property of Mr. C. H. Beeson, "Leyburn", near Gunnedah, N.S.W. The location of each plant was mapped approximately at the time of collection and a number given. In Table 3, these isolations are listed in the same order of proximity as in the field.

An isolation was made from a single nodule of each plant which had been surface-sterilized in the usual way with mercuric chloride, washed, crushed and stroked across plates of yeast-mannitol agar. The culture was then developed from a single, well-isolated colony and tested on potato, litmus milk, and for ability to produce nodules.

Antigen was prepared and tested as already described against representative antisera which had been prepared in connection with the earlier study of strains of *Rhizobium*

TABLE 3.
Location of Strains isolated from Medicago minima.

Location Number.	Description of Area.	Index to Serological Reactions.
MM.		
8	Virgin soil behind dam	h
9		g
10		f
11	Cleared 1936; under wheat 1936–40; grazed during 1941	e_1
12		e
13		i
14		i
5		e_1
6	Virgin soil on creek bank	a_2
7		a_1
1		e_2
2	Cleared 1932; wheat 1933-34; wheat and lucerne 1934;	g
3	lucerne died out 1936; grazed since 1934	g
4		i
15		i
16		b
17	Virgin soil, horse paddock	d
18		i
19		g
20		g

Note.—The symbol for index to serological reactions is obtained from the succeeding table.

TABLE 4.
Reactions of Cultures isolated from Separate Plants of Medicago minima.

	Reactions with Test Sera.							
	Flagellar.		Somatic.					
Location Number.	47 Ab.	27 bC.	47 I, III.	27 I, IV.	74 II, VI.	102 III, V.	66 II, VII.	Index to Serological Reaction.
7	±	3	3	2	–	–	–	a_1
6	2	3	3	±	–	–	–	a_2
16	2	4	–	–	–	–	–	b
12	±	3	2	–	–	–	3	c
17	1	3	–	–	–	–	–	d
5	3	±	4	3	–	–	–	e_1
11	3	±	3	3	–	–	–	e_1
1	3	±	1	4	–	–	–	e_2
10	4	±	–	–	2	–	–	f
2	3	±	–	–	–	–	–	g
3	3	±	–	–	–	–	–	g
9	3	±	–	–	–	–	–	g
19	3	±	–	–	–	–	–	g
20	3	–	–	–	–	–	–	g
8	–	±	3	2	–	–	–	h
4	–	–	–	–	–	–	–	i
13	–	–	±	–	–	–	–	i
14	–	–	±	–	–	–	–	i
15	–	–	–	–	–	–	–	i
18	–	–	–	–	–	–	–	i

Key to reactions: As in Table 1.

meliloti (Vincent, 1941). The results, all of which were checked by a repeated test, are given in Table 4. Flagellar (*H*) reactions are recorded for antisera 47 and 27 representing antigens Ab and bC respectively, and the somatic (*O*) for the five sera: 47, 27, 74, 102 and 66, which represent the seven somatic antibodies so far recognized within the species.

At least nine groups can be recognized amongst the twenty isolations of this experiment and that without counting probable sub-groups of *a* and *e* nor examining further cases listed as *g* and *i*, subdivision of which might occur if tested against additional antisera. Not only are the strains within the relatively-restricted area of a farm very heterogeneous, but there is also little if any relationship between directly neighbouring strains (see Table 3). As they occurred in the field the plants 9–14 were very close together yet their cultures represented at least five distinct serological groups.

It should be noted also that whereas the bulk (39/47) of the flagellar reactions previously recorded for this species of *Rhizobium* (Vincent, 1941) were of the *bC* type, in this case only five reacted in this way compared with nine reacting in the *Ab* fashion. Six cultures failed to react with either types of *H* antibody. In the latter case lack of *H* reactivity went along with observed motility and those which failed to react with both *H* and *O*, yet produced nodules on species of *Medicago*.

Experiment 2.—Isolations from Trifolium glomeratum.

Plants were collected from the property of Mr. W. W. Watson, "Woodbine", Tichborne, N.S.W. The location of each was recorded as in Exp. 1. The isolation and testing procedures were similar to those already described for strains from *Medicago minima*. Antisera were those developed against strains 36, 46, 61, 111 and 91 and used in a previous study of *Rhizobium trifolii* (Vincent, 1942), but it is only necessary to record results for *H* against 36(A) and 46(B) and for *O* against 36 (I), 61 (II) and 46 (III). Data are given in Tables 5 and 6.

TABLE 5.
Location of Strains isolated from Trifolium glomeratum.

Location Number.	Description of Area.	Index to Serological Reactions.
TG 1	Wheat stubble paddock	g_1
3	Wheat stubble paddock	a
4	Unbroken soil near 3	c
5	Oat paddock	g_2
6	Grass paddock	b_1
7		h
8	Wheat paddock	h
9		a
10		e_1
11	Wheat paddock	b_2
12		g_2
13	Near clover plots	e_1
14		d
15	Grassed	c
16		c
17		b_1
18	About house	e_2
19		h
20		f

Note.—The symbol for index to serological reactions is obtained from the succeeding table.

TABLE 6.

Reactions of Cultures isolated from Separate Plants of Trifolium glomeratum.

Location Number.	Reactions with Test Sera. Flagellar. 36 A.	46 B.	Somatic. 36 I.	61 II.	46 III.	Index to Serological Reactions.
3	4	—	4	—	—	a
9	4	—	4	—	—	a
6	3	—	—	3	—	b_1
17	3	—	—	3	—	b_1
11	3	—	—	1	—	b_2
15	4	—	—	—	—	c
16	3	—	—	—	—	c
4	3	—	—	—	—	c
14	3	3	—	—	—	d
10	—	3	—	—	±	e_1
13	—	2	—	—	—	e_1
18	—	1	—	—	—	e_2
20	—	—	2	—	—	f
1	—	—	—	—	1	g_1
5	—	—	—	—	4	g_2
12	—	—	—	—	3	g_2
7	—	—	—	—	—	h
8	—	—	—	—	—	h
19	—	—	—	—	—	h

Key to reactions: As in Table 1.

These results are very similar to those obtained with *Rhizobium meliloti* in Experiment 1. Although the number of testing sera is less, and therefore the chances of subdivision reduced, it has been possible to divide the nineteen cultures into at least eight groups. Some of these (viz., b, e, g, c and h) might be further divisible. As before, strains from neighbouring plants can be quite different and the same (or similar) type is likely to be picked up from widely separated parts of the farm.

Strains which failed to give an *H* reaction were definitely motile and all produced nodules on clovers.

Experiment 3.—Isolations from Trifolium repens.

Plants were collected from six localities spaced over the grounds of the University of Sydney. Three plants growing within a few feet of each other were taken from each location and an isolation made from a nodule of each plant as before. Tests were carried out against the same sera as were used in Experiment 2; relevant data are supplied in Tables 7 and 8.

At least six groups can be recognized, and additionally, for the same reasons as noted above, groups b, e, d and f might well subdivide further. Strains from three plants collected close to each other show some similarity, as for example 3·1 compared with 3·3 and possibly cases within 4, 5 and 6 localities. However, it must be noted that since the group *d* is a rather indeterminate one, the similarity may be superficial. In other cases isolations from the same locality are quite different from each other, and this is particularly so in the cases of 1 and 2. The fact that these were situated in an area used as a horse-yard might be responsible for the marked heterogeneity of the strains. The other areas were either lawns or recently built-up ground carrying only occasional plants.

TABLE 7.

Location of Strains isolated from Trifolium repens.

Location Number.	Index to Serological Reactions.
T.R.	
1·1	f
1·2	b_2
1·3	a
2·1	c
2·2	d
2·3	e_1
3·1	b_1
3·2	f
3·3	b_1
4·1	d
4·2	d
4·3	e_2
5·1	d
5·2	d
5·3	f
6·1	d
6·2	d
6·3	d

Note.—The symbol for index to serological reactions is obtained from the succeeding table.

TABLE 8.

Reactions of Cultures from Separate Plants of Trifolium repens.

	Reactions with Test Sera.					
	Flagellar.		Somatic.			
Location Number.	36 A.	46 B.	36 I.	61 II.	46 III.	Index to Serological Reactions.
1·3	3	–	2	±	±	a
3·1	3	–	–	2	2	b_1
3·3	2	–	–	2	2	b_1
1·2	3	–	–	2	±	b_2
2·1	3	–	–	2	–	c
2·2	2	–	–	–	–	d
4·1	3	–	–	–	–	d
4·2	3	–	–	–	–	d
5·1	3	–	–	–	–	d
5·2	3	–	–	–	–	d
6·1	3	–	–	–	–	d
6·2	3	–	–	–	–	d
6·3	3	–	–	–	–	d
2·3	–	–	2	–	–	e_1
4·3	–	–	1	±	–	e_2
1·1	–	–	–	–	–	f
3·2	–	–	–	–	–	f
5·3	–	–	–	–	–	f

Key to reactions: As in Table 1.

Experiment 4.—Strains of Rhizobium meliloti *obtained from Plants developed from Sterilized Seed sown in a Small Area.*

Sterilized seeds of *Medicago sativa*, *Medicago hispida* var. *denticulata* and *Melilotus alba* Desr. were sown within an approximate area of nine square yards of garden soil (Merrylands, N.S.W.). Isolations were made from separate plants, and cultures were tested against the same sera as were used in Experiment 1. All necessary data are given in Table 9.

TABLE 9.
Reactions of Cultures from Sown Medicago *spp. and* Melilotus alba.

Location.	Host.	Reactions with Test Sera. Flagellar. 47 Ab.	27 bC.	Somatic. 47 I, III.	27 I, IV.	74 II, VI.	102 III, V.	66 II, VII.	Index to Serological Reaction.
4	M.S.	—	4	2	3	—	—	—	a
4	M.H.D.	—	4	3	4	—	—	—	a
4	Mel.A.	—	4	3	4	—	—	—	a
5	M.S.	—	3	2	3	—	—	—	a
5	M.H.D.	—	4	2	3	—	—	—	a
5	Mel.A.	—	4	3	2	—	—	—	a
7	M.S.	—	3	2	4	—	—	—	a
7	M.H.D.	—	3	2	3	—	—	—	a
8	M.H.D.	—	3	2	4	—	—	—	a
8	Mel.A.	—	4	2	3	—	—	—	a
2	M.S.	—	4	1	—	±	2	1	b
7	Mel.A.	—	3	—	—	—	—	—	c

Key to reactions: As in Table 1.
Key to host plant: M.S. = *Medicago sativa*; M.H.D. = *Medicago hispida* var. *denticulata*; Mel.A. = *Melilotus alba*.

Within this small area there is evidently a fair degree of homogeneity. It should be noted that the reaction here designated as *a* is of the same kind as that shown by strain 27 itself isolated from a plant growing within a few yards of the garden plot used for this experiment. Cultures with similar reactions were commonest amongst the isolations tested in the first paper of this series.

B. *Strains isolated from the Same Plant.*

Experiment 5.—Comparisons of Cultures isolated from Pairs of Nodules.

In the cases of twelve plants of *T. repens* (selected at random) used in Experiment 3, a second nodule was used for isolation. The reactions of the second culture are compared with those of the first in Table 10. The second isolation is indicated by an extra digit—e.g., 11·1 is to be compared with 1·1, 12·1 with 2·1, etc.

The extreme right-hand column of the table includes an estimate of the agreement between members of each pair of nodules. In five cases the difference between them is quite definite and in at least one other case a difference is probable. In cases classed as possibly alike, a more detailed examination would be required to establish serological identity. These results show that it is not uncommon for an individual plant growing in this area to have nodules on it developed from different strains of organism. This is supported by results reported in Table 11 in connection with the next experiment; only in one out of four plants were all isolations from replicate nodules the same.

Experiment 6.—Tests of Cultures developed from the Same Nodule.

In the case of four plants of *T. repens* isolations were made from more than one nodule per plant, and additionally a number of single colony pickings was made from the growth that developed for each nodule. The plates from which the preliminary pickings were made were kept in a refrigerator so that in the cases of pairs of nodules which showed definite differences for the one plant, more cultures could be developed. The number of colonies tested in each case is shown in Table 11.

TABLE 10.
Cultures isolated from Pairs of Nodules.

Culture.	Reactions with Test Sera. Flagellar. 36 A.	46 B.	Somatic. 36 I.	61 II.	46 III.	Comparison.
1·1	–	–	–	–	–	Unlike.
11·1	2	–	–	2	–	
1·2	3	–	–	2	±	Probably unlike.
11·2	2	–	1	1	–	
1·3	3	–	2	±	±	Unlike.
11·3	2	–	–	–	–	
2·1	3	–	–	2	–	Unlike.
12·1	2	–	–	–	–	
2·2	2	–	–	–	–	Possibly alike.
12·2	2	–	–	1	–	
2·3	–	–	2	–	–	Possibly alike.
12·3	–	–	3	±	±	
3·1	3	–	–	2	2	Unlike.
13·1	2	–	–	2	–	
3·3	2	–	–	2	2	Unlike.
13·3	±	–	±	–	–	
4·2	3	–	–	–	–	Possibly alike.
14·2	2	–	–	1	–	
5·1	3	–	–	–	–	Possibly alike.
15·1	1	–	–	–	–	
5·2	3	–	–	–	–	Possibly alike.
15·2	2	–	–	–	–	
6·2	3	–	–	–	–	Possibly alike.
16·2	±	–	–	–	–	

Key to reactions: As in Table 1.

TABLE 11.
Tests of Cultures from the Same Plant and Same Nodule.

Plant.	Nodule.	No. of Colonies Tested.	Reactions with Test Sera. Flagellar. 36 A.	46 B.	Somatic. 36 I.	61 II.	46 III.
A	1	4	3	–	±	–	–
A	2	4	3	–	±	–	–
B	1	6	–	–	–	3	–
B	2	6	–	–	–	–	–
C	1	11	3	–	–	±	–
C	2	2	3	–	–	–	–
C	3	3	3	–	–	–	–
C	4	13	–	–	–	2	2
D	1	2	3	–	–	–	–
D	2	12	3	–	–	3	–
D	3	3	3	–	–	2	–
D	4	7	3	–	–	–	–

Key to reactions: As in Table 1.

Altogether seventy-three single colony pickings have been made from twelve nodules, including those from plants known to harbour more than one strain in different nodules. In no case has more than one serological type been found within the nodule. This homogeneity contrasts strongly with the heterogeneity that has been established amongst plants growing close together and even in different nodules of the same plant.

Discussion.

From the results reported in this paper it would seem that not only is there considerable heterogeneity within the species *Rhizobium meliloti* and *Rhizobium trifolii* amongst cultures collected from different districts, but that those coming from relatively close areas and even from different nodules of the same plant can fall into distinct serological groups. It will be difficult, therefore, to form any estimate of the characteristics of the rhizobial population of a given area without the collection and testing of a large number of cultures.

In striking contrast to this state of affairs is the homogeneity existing within the single nodule. This agrees with the report of Dunham and Baldwin (1931) and indicates that some factor is operating to restrict the development within a single nodule, at least largely, to a single strain. It would be of considerable interest to know whether this factor operates (*a*) outside the root-hair, controlling the nature of the intimate rhizobial population, (*b*) at the time of invasion by favouring the entry of one kind out of a mixed population, or (*c*) within the nodule, causing the development of just the one strain and the overgrowth and inhibition of others. Recently Nicol and Thornton (1941) have postulated competition between strains outside the plant as a major factor in determining the extent to which one particular strain will gain entry rather than another to which the plant is exposed at the same time. However, in view of the occurrence of different strains on neighbouring nodules, this will scarcely explain the purity of culture within the nodule. A recent report by Jensen (1941) of rhizobia obtained from nodules of *Medicago* spp. proving to be *Rhizobium trifolii* (able to nodulate *Trifolium* but not *Medicago*) is somewhat surprising in view of our results.

Summary.

The agglutination reaction has been used to analyse strains of *Rhizobium* (*a*) occurring on different plants growing within a well-defined area, (*b*) obtained from different nodules on the same plant, and (*c*) found within the one nodule. Experience of other workers and our own results show that the test is reliable and reflects some fundamental characteristic of the bacterial cell which is relatively constant. Good agreement has been obtained (*a*) with sera from different individual animals, (*b*) after prolonged laboratory cultivation, and (*c*) after passage through a host plant.

Twenty cultures of *Rhizobium meliloti*, obtained from separate plants of *Medicago minima*, all of which were growing within the same farm area, contained at least nine distinct serological groups. Furthermore, there was no evident relationship between location in the field and affinity in observed reaction. Similar tests with *Rhizobium trifolii*, both from *Trifolium glomeratum* and *T. repens*, showed at least eight strains in nineteen and six in eighteen cultures respectively. Twelve cultures of *Rhizobium meliloti* from a much smaller area showed three types, although one of these predominated.

When cultures developed from pairs of nodules—each pair from the same plant—were compared, it was found that in five cases the members of the pair were definitely unlike; in one case a difference was probable, whilst the remainder were possibly alike.

In contrast to this heterogeneity, replicate single-colony pickings from material developed from the same nodule gave consistently the same result. No case of difference within the nodule was found with cultures developed from seventy-three colonies representing in all ten nodules from four plants, although it was known that this material showed differences between nodules.

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